Sperm investment and alternative mating tactics in bluegill sunfish (*Lepomis macrochirus*)

Bryan D. Neff,[a] **Peng Fu,**[b] **and Mart R. Gross**[b]
[a]Department of Biology, University of Western Ontario, London, Ontario N6A 5B7, Canada, and
[b]Department of Zoology, University of Toronto, 25 Harbord Street, Toronto, Ontario M5S 3G5, Canada

Although alternative mating tactics are found in males of many species, little is known about tactic-specific adaptations to sperm competition and the mechanism by which fertilization success is obtained. We now report on the sperm investment patterns of males that use alternative mating tactics in bluegill sunfish (*Lepomis macrochirus*). Bluegill males are characterized by three alternative mating tactics: parentals, sneakers, and satellites. Parentals delay maturation and adopt a courting and guarding tactic, whereas sneakers and satellites mature precociously and, respectively, use sneaking and female mimicry to steal fertilizations from parentals. We found differences among the tactics in testes weight, sperm longevity, and ejaculate sperm density and competitiveness. Parentals had the largest testes, but the smallest relative to their body weight. Satellites had intermediate-sized testes, and sneakers had the smallest testes, but the largest relative to their body weight. Within each tactic, there was a positive relationship between testes weight and body weight, but the exact relationship differed among the tactics and could not be attributed solely to allometry. Parentals had longer-lived sperm compared with that of both sneakers and satellites. Ejaculate sperm density was greatest in sneakers, satellites were intermediate, and parentals had the lowest. Competition experiments involving equal volumes of ejaculate showed that fertilization success increased linearly with sperm number, consistent with a rafflelike process. However, independent of sperm number, parental sperm were superior at fertilizing eggs. We interpret these sperm investment patterns in relation to differences in sperm competition risk, number of mating opportunities, and alternative investment options. *Key words:* bluegill, fish, sperm competition, strategies, tactics. *[Behav Ecol 14:634–641 (2003)]*

In many species in which males must compete for mating access to females, less competitive males may adopt alternative mating tactics (Gross, 1996). Alternative mating tactics are especially widespread in the fishes, likely because of their indeterminate growth, which results in large variation in body size; the prevalence of external fertilization in an aqueous medium, which facilitates sperm competition; and the frequency of paternal care, which adds to the benefit of an alternative non-caring tactic (Taborsky, 1994, 1998; see also Neat and Locatello, 2002). Two mating tactics are typically adopted by males: a bourgeois tactic used by males that defend and attempt to monopolize females, and a parasitic tactic used by males that attempt to exploit bourgeois males (Taborsky, 1997). Bourgeois and parasitic males often engage in sperm competition whereby their sperm must compete to fertilize the same egg(s). Parker (1990a, 1998) proposed the sneak-guard model of sperm competition in which certain males, the guards, are paired permanently to females, whereas other males, the sneaks, specialize at stealing fertilizations. He predicted that when males have exclusive access to eggs, sperm investment should increase linearly with the number of eggs or mating opportunities available to the male. However, when there is sperm competition, males may invest considerably more in sperm than would be required to fertilize the eggs in the absence of competition. As such, sneaks should be specialized sperm competitors because they typically experience much higher levels of sperm competition.

Trade-offs with alternative investment options such as mate guarding (exclusion of competing males) can also influence sperm investment patterns. Alonzo and Warner (2000) have shown that individuals may not invest in sperm when faced with a higher risk of sperm competition if they can instead invest in mate guarding that effectively excludes sneaks. Thus, guards may respond to an increased risk of sperm competition either by increasing their sperm competitiveness or by excluding sneaks and reducing the number of times their sperm must compete. Consequently, mating opportunities, sperm competition risk, and alternative investments must all be considered when examining sperm investment patterns.

In this article, we investigate the sperm characteristics and investment patterns of male bluegill sunfish (*Lepomis macrochirus*). Male bluegill have a discrete polymorphism in life histories termed "parental" and "cuckolder" (Gross, 1982). Parentals are specialized nest and mate guarders, while cuckolders are specialized sperm competitors that gain entry into parental nests as either "sneakers" or "satellites." In Lake Opinicon (Ontario, Canada), parentals delay maturation to age 7 years (Gross, 1982). Cuckolders mature precociously at age 2 years; when small (age, 2–3 years), as sneakers they use a sneaking, ambush tactic, and when large (age, 4–5 years), as satellites they use female mimicry to obtain access to spawning females. Males therefore are characterized by three mating tactics: parental, sneaker, and satellite. Although only about 20% of juvenile males mature precociously as cuckolders, because of differential survivorship to age of maturation, cuckolders represent about 80% of breeding male population (Gross, 1982).

During the breeding season, parentals compete for nesting sites within colonies and construct nests within the substrate. Colonies are densely packed with rim-to-rim nests numbering up to several hundred (Cargnelli and Gross, 1996). Females join the colony as a school and select males by entering their nests. Females are protracted spawners, releasing only about 10–30 eggs per "dip," but may remain within a male's nest until several thousand eggs are released. Parentals actively defend their nest from intrusions by sneakers but typically

readily attempt to spawn with satellites, presumably because they are deceived that they have two females (Gross, 1982; Neff and Gross, 2001). Spawning lasts about 6–12 h, after which only the parentals remain in the colony to provide sole care for the developing young in their nest. The parental care period lasts 7–10 days, during which a parental does not spawn again.

Sperm competition occurs when a sneaker or satellite intrudes on a mating between a parental and female. The parental is virtually always present during an intrusion, and therefore, cuckolders experience sperm competition nearly 100% of the time. Because cuckolders successfully intrude in only about 20% of female dips (Gross, 1982; Gross and Charnov, 1980), parentals experience a considerably lower risk of sperm competition. Leach and Montgomerie (2000) compared sperm from sneakers with those parentals and found that sperm density in stripped ejaculates from sneakers is higher, and although sperm from sneakers appears to swim slower, it is of similar length and longevity. We have also shown that cuckolders are superior sperm competitors, as sneakers and satellites fertilize 89% and 67%, respectively, of the eggs when in direct competition with parentals (Fu et al., 2001). Furthermore, females seem to prefer to mate with cuckolders as they release about three times as many eggs when spawning in the presence of a cuckolder (Fu et al., 2001).

Sperm investment by males with alternative mating tactics has been studied in only a few other systems (for review, see Birkhead and Møller, 1998). In general, these studies have shown that both increased mating opportunities and increased risk of sperm competition lead to increased investment into sperm production. In fishes, this investment has most commonly taken the form of larger relative testes weight and increased sperm density and motility. Few differences, however, have been found in sperm morphology. For example, in Atlantic salmon (*Salmo salar*), Gage et al. (1995, 1998) found that precocial parr (sneaks) invest more in total spermatogenesis (as measured by gonadal somatic index) compared with anadromous males (guards). Parr sperm also live longer but do not appear to differ in morphology (see also Vladić and Järvi, 2001). Similar results have been found in European ocellated wrasse, *Symphodus ocellatus* (Alonzo and Warner, 2000); bluehead wrasse, *Thalassoma bifasciatum* (Schärer and Robertson, 1999); corkwing wrasse, *Symphodus melops* L. (Uglem et al., 2001); three-spined stickleback, *Gasterosteus aculeatus* (de Fraipont et al., 1993); and the peacock blenny, *Salaria pavo*, and Azorean rock-pool blenny, *Parablennius sanguinolentus parvicornis* (Oliveira et al., 2001). One exception has been reported in the triplefin blenny, *Axoclinus nigricaudus*, in which there was no difference in the relative investment into spermatogenesis between territorial and nonterritorial satellite males (Neat, 2001).

In this article, we investigate sperm characteristics and investment patterns in the three bluegill mating tactics—sneakers, satellites and parentals—and discuss these patterns in relation to mating opportunities, risk of sperm competition, and alternative investments. Specifically, for each of the three tactics, we measure (1) mating opportunities, (2) sperm competition risk, (3) absolute and relative investment into testes, (4) sperm longevity and density, and (5) ejaculate competitiveness.

METHODS

Behavioral observations at colonies and collections of breeding bluegill, including sneakers, satellites, parentals, and females, were made in Lake Opinicon (near Kingston, Ontario: 44°16′ N and 76°30′ W) during the breeding seasons (May–July) of 1984, 1992, 1993, 1996, and 1999 following the methods described by Gross (1980, 1982). Statistical calculations were performed with SPSS (version 10.0). All mean values are expressed with ±1 SD, and all statistical tests were performed with two-tailed levels of significance. All ANOVAs were performed assuming an unbalanced design with type III sum of squares with Tukey's post hoc analysis. Slope comparisons between linear regressions were performed using Student's t tests. Proportional data were arcsine square-root transformed to remove mean-dependent variance (Zar, 1999).

Sperm competition risk

Mating observations were opportunistically made at 36 nests in five colonies during three spawning bouts in 1999 (see Fu et al., 2001). Female dips were counted, and the number and types of males that partook in spawning were recorded. Sperm competition risk for each mating tactic was calculated as the proportion of dips that were in the presence of an additional male. Within parentals, we used correlation to investigate the relationship between body weight and risk.

Mating opportunities

From the mating observations made at the 36 nests in 1999, each spawning cuckolder was visually classified as small or large sneaker, or small or large satellite (based on their body length and behavior; see Gross, 1982). Using the age/size class data presented in Gross (1982), χ^2 analysis was used to determine if the observed number of intrusions differed from those expected under the assumption that there is no difference in the number of mating opportunities per individual across the four size categories. The overall intrusion frequency for each category was calculated as the number of successful intrusions divided by the total number of dips observed. These frequencies were then converted to per individual frequencies by dividing by the relative number of individuals in each category.

For parentals, because not all spawning was observed at each of the 36 nests, we quantified the total number of eggs obtained by each male using egg scores (see Cargnelli and Gross, 1996). Egg scores are a rank between one (few eggs) and five (many eggs) and are assigned based on the visually assessed percent coverage of eggs within the nest. As an example, a "one" represents 20% coverage. This rank highly correlates with the actual number of eggs in a nest (Claussen, 1991). The egg scores were assumed to reflect the number of mating opportunities for each parental because females rarely spawn in the absence of the parental. The weight of each parental was also measured. These data were combined with egg score and parental weight data from an addition 603 nests collected from two other years (1992, 1993). Thus, for parental mating opportunities, we had data from 639 males. Spearman nonparametric correlation was used to assess the relationship between parental weight and the number of mating opportunities as measured by the egg scores.

The number of eggs spawned within a bout that were available to individual sneakers or satellites relative to parentals was calculated based on the overall cuckolder intrusion frequency, the relative number of eggs released by females in the presence of a cuckolder versus when only a parental is present, and the relative number of breeding cuckolders to parentals in the population.

Testes investment

Forty-five sneakers, 27 satellites, and 69 parentals were collected at colonies at the onset of spawning in 1999. Total body weight and testes weight were obtained from each male. These data were log-transformed, and testes weights were

regressed onto body weights (Stockley et al., 1997). The analysis was performed for each tactic, as well as for sneakers and satellites combined and for all males. The slope of each regression was compared to 0.75, which might be expected when there is allometric growth (Stockley et al., 1997; Taborsky, 1998).

The gonadal somatic index (GSI) for each tactic was calculated as GSI = testes weight/total body weight, and values were compared among the three tactics by using ANOVA. Following the method of Tomkins and Simmons (2002), we also used ANCOVA to investigate differences in testes weight among the tactics, using log body weight as the covariate. Following the method of Taborsky (1998), we used regressions to examine the relationship between GSI and body weight. These latter regressions were interpreted with caution because body weight appears in both the dependent and independent variables, which can lead to spurious correlations (Zar, 1999). Furthermore, because gonad weight might scale to body weight by a factor of 0.75, negative relationships could be the result of allometric growth and not reduced relative investment in sperm (for further discussion, see Taborsky, 1998). Therefore, for negative relationships we also computed a modified GSI by raising gonad weight to the power of 4:3 to account for potential allometry before dividing by body weight.

Sperm longevity

Equal volumes of milt (50 µl) were collected in 1996 from seven sneakers, six satellites, and 10 parentals by applying gentle pressure to the gonad region and collecting the milt excreted with graduated syringes. The milt samples were then placed on glass slides, and the spermatozoa were "dilution-activated" (Scott and Baynes, 1980) with lake water (100 µl) and observed under a compound light microscope with ×100 magnification at room temperature. Sperm longevity was estimated as the time from activation until approximately 80% of the spermatozoa within the field of view were no longer motile (showing forward movement). Because our criterion was subjective, measurements were repeated four times for each male, twice by each of two observers. Repeatability was examined within observers by using correlation between the two measurements taken for each fish. These two measurements were then averaged, and the repeatability across observers was examined by using correlation between the two means for each fish. Sperm longevity among mating tactics was then examined by using ANOVA.

Sperm density

A sample of 10 µl of free-flowing milt was collected from each of 22 sneakers, 26 satellites, and 76 parentals in 1984. From each sample, 2 µl was diluted in 1 ml of water and thoroughly mixed. Six microliters of the dilution was then injected into a Bright-line hemacytometer (American Optical), and the sperm were counted in five 0.2 × 0.2-mm grids under a compound light microscope at ×400 magnification. Sperm density was calculated by multiplying the mean sperm count per grid by the dilution factor and volume. The testes of the males were then removed and weighed. The weight change in testes owing to the removal of milt was negligible. Density was compared among the tactics with ANOVA and the relationship between sperm density and testes weight was examined by using linear regression.

Ejaculate competitiveness

We tested ejaculate competitiveness by using a two-factor experiment involving both sperm number and tactic-specific competitiveness. Milt and eggs were stripped from two sneakers, two satellites, four parentals, and three females in 1996. Equal volumes of milt (100 µl) from a cuckolder and a parental were mixed and dilution-activated, and the paired milt was released over a sample of 30–100 eggs from one of the females. There was a total of 16 trials involving all combinations of a cuckolder and parental male (four cuckolders × four parentals). The containers with milt and eggs were placed in an aquarium of lake water maintained at ambient temperature for incubation. The embryos were reared for 7 days and then preserved in ethanol. Egg hatching and rearing was successful in 12 of the 16 trials. DNA was isolated from the adults and fry, and microsatellite DNA fingerprints were obtained by using multiplex genotyping (Neff et al., 2000). Up to five primer sets (published in Colbourne et al., 1996; Neff et al., 1999, 2000) were simultaneously amplified, and all competing parental and cuckolder offspring were unambiguously identified by using exclusion methods (see Chakraborty et al., 1988). An index of the relative paternity in each competition trial was calculated from $\ln(Pat_c + 1) - \ln(Pat_p + 1)$, where Pat is the paternity (expressed as a proportion) of the cuckolder (c) or parental (p). Thus, positive values represent trials in which the cuckolder fertilized a majority of the eggs, and negative values represent trials in which the parental fertilized a majority. The number of sperm in the 100 µl samples were calculated based on each male's testes weight and the relationships derived from the sperm density data (above). From these values, an index of the relative number of sperm used in each trial was calculated from $\ln(NS_c) - \ln(NS_p)$, where NS is the number of sperm (in units of a million) for the cuckolder (c) and parental (p). Linear regression was used to examine the relationship between relative sperm number and relative paternity.

RESULTS

Sperm competition risk

In total, 7471 dips were observed in the nests of parentals. Cuckolders attended 766 (10.3%), and the parental was alone in the remainder. Cuckolders always experienced sperm competition because the parental was present every time the cuckolder intruded. Thus, cuckolders had a 100% risk of sperm competition. Parentals, however, spawned alone with the female in 6705 (89.7%) dips and, therefore, experienced sperm competition in only 10.3% of dips. Gross (1982) found that cuckolders intruded in 20% of female dips (see also Gross and Charnov, 1980). Thus, parentals may experience sperm competition 10–20% of the time. We also found that larger parentals (as measured by weight) experienced lower intrusion rates and, hence, a lower risk of sperm competition ($r = -.44$, $p < .05$, $N = 36$).

Mating opportunities

The mating opportunities for cuckolders varied with their size and tactic. Small sneakers, large sneakers, small satellites, and large satellites attended 6.0% (450 dips), 1.3% (98), 1.2% (92), and 1.7% (126) of the 766 dips, respectively. From Gross (1982), the ratios of the number of cuckolders in each of these four size categories are 1:0.42:0.40:0.07. Assuming each cuckolder had equal mating opportunities, then the number of dips attended in each size category should have been 405, 170, 162, and 29, respectively (e.g., 405 = 766 × 1/1.89). These values differed significantly from those observed ($\chi^2 = 390$, df = 1, $p < .001$). Although this result is highly significant, some caution should be taken because each dip was not statistically independent, which would require observing different males and females for each dip. Nevertheless, the
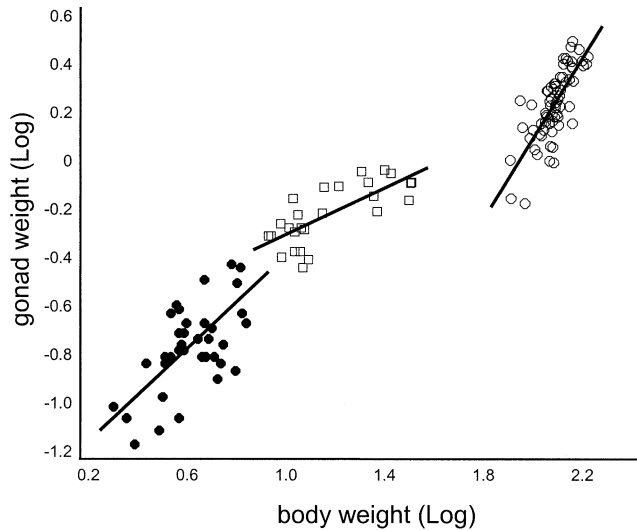
Figure 1
Relationship between testes weight and body weight for sneakers (closed circles), log(*testes weight*) = [0.95 × log(*body weight*) − 1.39]; satellites (open squares), log(*testes weight*) = [0.48 × log(*body weight*) − 0.81]; and parentals (open circles), log(*testes weight*) = [1.64 × log(*body weight*) − 3.26]. The slopes are significantly different, and all differ from 0.75 ($p < .05$ for each comparison).

data indicate that the mean number of mating opportunities *per individual* differed across the four categories. The relative intrusion frequencies were 1:0.52:0.50:4.2. Large sneakers and small satellites (i.e., intermediate-sized cuckolders) were the least successful, obtaining about half the number of mating opportunities as that of small sneakers and only 12% as many as that of large satellites. Large satellites were the most successful, obtaining more than four times the number of mating opportunities as that of small sneakers. Satellites overall obtained about three times as many matings per individual as that of sneakers ([0.50 + 4.2]/[1 + 0.52] = 3.1).

There appeared to be a negative relationship between body size and mating opportunities in sneakers, and a positive relationship in satellites, albeit these relationships were based on qualitative, categorical body size measurements (small versus large). Within parentals, there was a weak positive relationship between body weight and the number of mating opportunities ($\rho = 0.15$, $p < .001$, $N = 639$).

Given that cuckolder intrusion rates are 10–20% and assuming that females release about three times more eggs in the presence of cuckolders, cuckolders have access to 25–43% of the total eggs spawned within a bout (e.g., 25% = [0.1 × 3]/[0.1 × 3 + 0.9 × 1]). Because there are approximately four times as many breeding cuckolders as parentals, on an individual basis, cuckolders have access to only 6–11% as many eggs as parentals. Furthermore, given that satellites individually obtain three times as many matings as those of sneakers, satellites on average have access to 4–8% of the eggs and sneakers have access to the remaining 2–3%.

Testes investment

Testes weight differed significantly among parentals (1.82 ± 0.56 g), satellites (0.62 ± 0.19 g), and sneakers (0.20 ± 0.10 g) (ANOVA: $F_{2,144} = 249.9$, $p < .001$). Satellite and sneaker testes were 34% and 11%, respectively, the weight of parental testes. The GSI of sneakers (3.66 ± 1.45) and satellites (3.74 ± 1.06) did not differ (ANOVA: $F_{2,144} = 106.4$, $p = .93$), but both were significantly greater than that of parentals (1.32 ± 0.29) (Tukey's post hoc: $p < .001$ for both). The ANCOVA

analysis revealed highly significant effects of tactic, the covariate log body weight, and the interaction term ($F_{5,144} = 465.8$, $p < .001$ for each). Analogous results were found when body weight less gonad weight (referred to as soma mass in Tomkins and Simmons, 2002) was used as the covariate. The significant interaction term indicates that the allometry differs among the tactics. Unfortunately, it also makes the interpretation of the main effect difficult. We failed to rectifying the problem by adding or subtracting 1 SD from the covariate of one tactic as suggested by Tomkins and Simmons (2002). Alternatively, Tomkins and Simmons suggest that the slope of only one of the tactics be used in the covariance adjustment. However, given that all three slopes differed significantly, it seemed arbitrary as to which slope to select.

For parentals, there was a positive linear relationship between testes weight and body weight ($r^2 = .58$, $p < .001$, $N = 69$) (Figure 1) and between GSI and body weight ($r^2 = .18$, $p < .001$, $N = 69$) (Figure 2a). A similar relationship was found between the testes weight and body weight of sneakers ($r^2 = .48$, $p < .001$, $N = 45$) and satellites ($r^2 = .53$, $p < .001$, $N = 27$) (Figure 1). However, sneaker GSI and body weight were unrelated ($r^2 = .004$, $p = .68$, $N = 45$) (Figure 2b), and there was a negative relationship between satellite GSI and body weight ($r^2 = .47$, $p < .001$, $N = 27$) (Figure 2c). This latter relationship remained significant when the modified GSI that corrects for potential allometric growth was used (data not shown). Thus, the negative relationship in satellites can not be attributed to allometry alone.

Analyzing data from all cuckolders, there was a positive linear relationship between the logs of gonad weight and body weight ($r^2 = .82$, $p < .001$, $N = 72$), but the slope of .90 was significantly greater than .75 ($p < .05$). Across all males, there was also a positive linear relationship between these weights ($r^2 = .91$, $p < .001$, $N = 141$). In this case, the slope of the regression was .66, which was significantly less than .75 ($p < .05$).

Sperm longevity

There was high repeatability between the two measurements taken by each observer (observer 1: $r = .70$, $p < .001$, $N = 23$; observer 2: $r = .69$, $p < .001$, $N = 23$), and there was high consistency between the two observers ($r = .72$, $p < .001$, $N = 23$). Based on our 20% motile criterion, parental sperm lived 40% longer than did the cuckolder sperm (parentals, 159 ± 30 s; sneakers, 109 ± 19 s; satellites, 120 ± 33 s; ANOVA: $F_{2,23} = 7.66$, $p = .003$) (Figure 3a). There was no significant difference between sneakers and satellites (Tukey's post hoc: $p = .75$).

Sperm density

In equal volumes of ejaculate, cuckolders had more sperm than did parentals. The density of sperm produced by satellites exceeded that of sneakers, which in turn exceeded that of parentals (satellite, $2.0 \times 10^6 \pm 0.4 \times 10^6$ per mm^3; sneaker, $1.4 \times 10^6 \pm 0.2 \times 10^6$ per mm^3; parental, $0.9 \times 10^6 \pm 0.1 \times 10^6$ per mm^3; ANOVA: $F_{2,124} = 290.25$, $p < .001$) (Figure 3b). There was no relationship between sperm density and testes weight in parentals ($r^2 = .01$, $p = .29$, $N = 76$) (Figure 4a), but sperm density was positively related to testes weight in cuckolders ($r^2 = .57$, $p < .001$, $N = 48$). Sneakers and satellites had similar relationships (sneakers: $r^2 = .26$, $p = .02$, $N = 22$; satellites: $r^2 = .50$, $p < .001$, $N = 26$) (Figure 4b).

Ejaculate competitiveness

Relative cuckolder sperm number was positively related to the proportion of eggs that cuckolders fertilized ($r = .77$, $p = .004$,
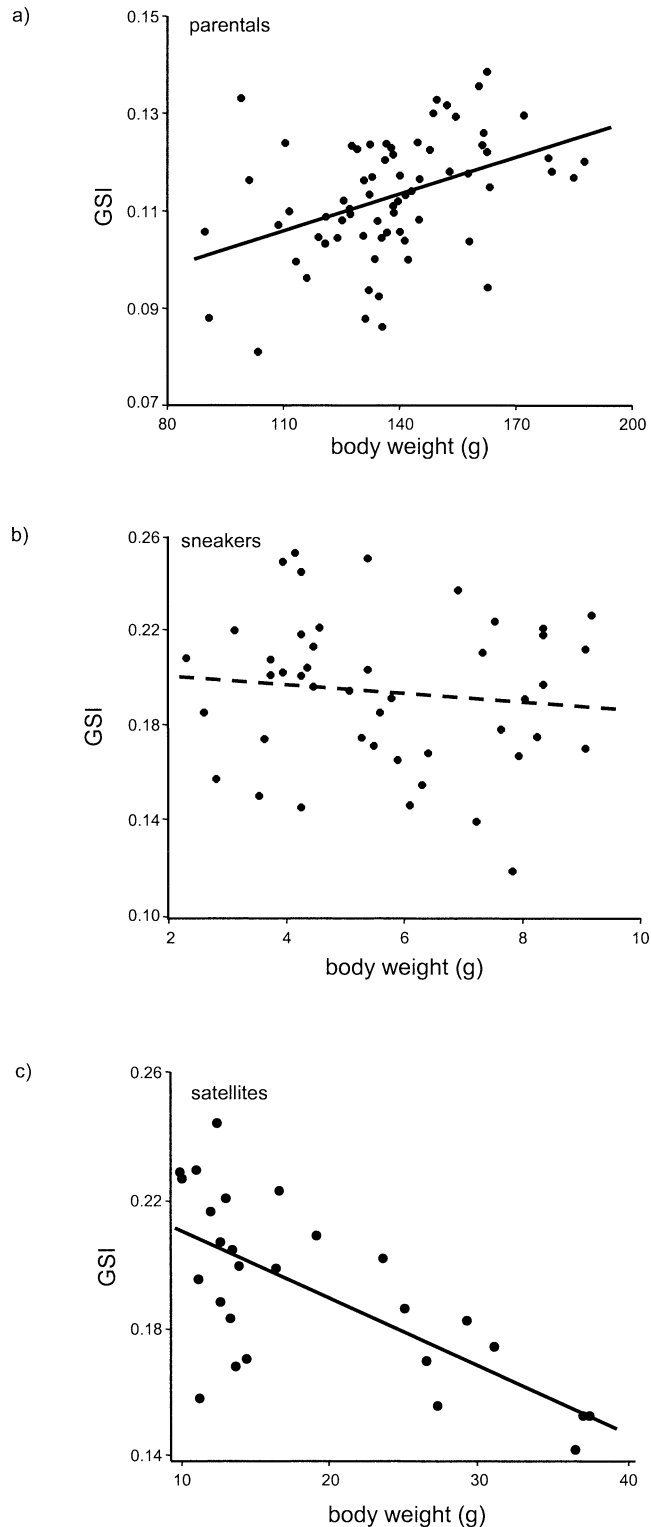
a)

b)

c)

Figure 2
Relationship between GSI and body weight for parentals (a), GSI = [0.00027 × body weight + 0.077]; sneakers (b), p = ns; and satellites (c), GSI = [−0.00225 × body weight + 0.234]. GSI is arcsine square-root transformed.

N = 12) (Figure 5). Thus, increasing sperm number increased fertilization success under competition. Although each trial had more sperm from cuckolders compared with parentals (because cuckolders have higher sperm density and equal
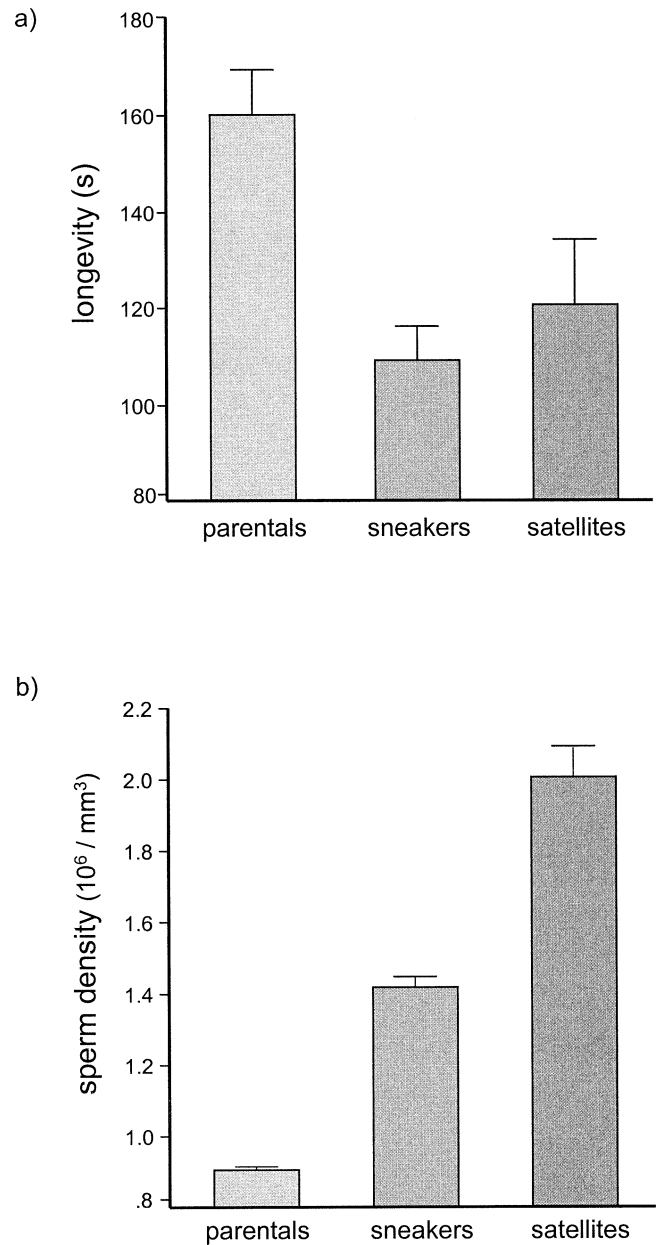
a)

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Figure 3
Variation among the three mating tactics in sperm longevity (a) and ejaculate sperm density (b). Error bars indicate standard errors.

volumes of ejaculate were used), cuckolders did not always fertilize more eggs.

DISCUSSION

We found significant differences in the sperm investment patterns among individuals from the three mating tactics in bluegill sunfish (sneakers, satellites, and parentals). We consider these investment patterns in relation to sperm competition risk, the number of mating opportunities, and alternative investment options.

Among the mating tactics, overall differences in the investment in sperm production relates to the risk of sperm
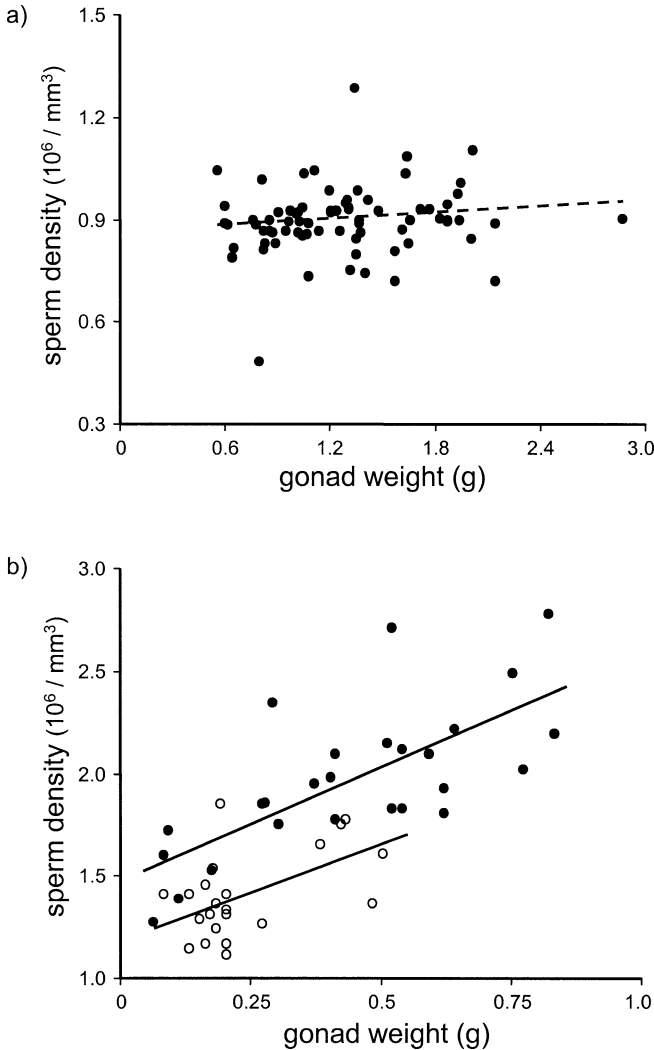
a)

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Figure 4
The relationship between ejaculate sperm density and testes weight: (a) parentals, $p = $ ns; and (b) sneakers (open circles), sperm/mm^3 = $0.88 \times 10^6 \times$ testes weight + 1.2×10^6, and satellites (closed circles), sperm/mm^3 = $1.1 \times 10^6 \times$ testes weight + 1.5×10^6.

Figure 5
Relationship between relative paternity and relative sperm number of parentals and cuckolders under direct sperm competition: relative paternity = [7.4 × relative sperm number − 3.7]. The dashed line represents equal paternity. Below the line, parentals have higher paternity, and above the line, cuckolders have higher paternity. Equal paternity occurs when cuckolders have approximately 1.7 times more sperm than do parentals in the milt mixture ($e^{0.51} = 1.7$).

competition and the number of mating opportunities. Sneakers and satellites almost always experience sperm competition, whereas parentals experience it only 10–20% of the time (this study; Gross, 1982). Consistent with Parker's (1990a, 1998) prediction, we found that cuckolders (both sneakers and satellites) invest about three times more of their somatic tissue into testes (GSI) compared with that of parentals. Individual sneakers and satellites have access to 2–3% and 4–8%, respectively, as many eggs as do parentals, and their testes weigh 11% and 34% as much as parental testes. Thus, across the three mating tactics, testes weight increases with the number of mating opportunities. However, both sneakers and satellites have testes that are roughly five times larger than would be predicted solely based on the number of mating opportunities, likely relating to the increased sperm competition experienced by these males (Parker, 1990a, 1998). Because we included the relative number of breeding cuckolders to parentals in our calculations of mating opportunities, these differences can not be attributed to the fact that cuckolders provide no care for their offspring and are thus free to mate again sooner than parentals.
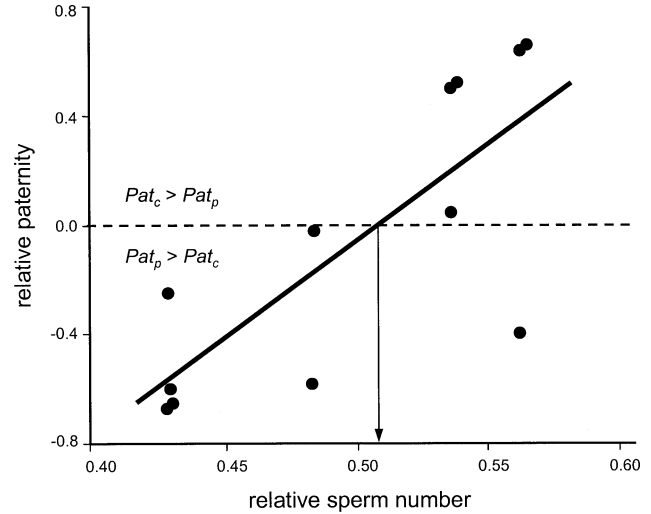
The differences in sperm investment among tactics might also relate to trade-offs with alternative investments such as territory defense and mate guarding. For example, larger parentals can obtain superior positions in the center of spawning colonies and thereby reduce their risk of sperm competition (Gross, 1980; Neff, 2000). Conversely, cuckolders always experience sperm competition regardless of their body size. Thus, parentals instead may invest proportionately more in body size compared with that of cuckolders, and thereby indirectly invest in sperm competition by reducing the number of times their sperm must compete with a cuckolder's (see also Alonzo and Warner, 2000). We can reject the notion that the differences we observed in testes weight among the tactics are owing simply to allometry because the slope of the relationship between testes weight and body weight differed in each tactic, and across all males, it was significantly different from the expected 0.75 (Stockley et al., 1997; Taborsky, 1998).

Within each mating tactic, investment in sperm production made by individuals generally relates to the number of mating opportunities but not to the risk of sperm competition. Within parentals, larger males obtain more mating opportunities and have absolutely larger testes. These males also invest proportionately more in their testes, as measured by GSI. However, larger parentals actually experience a lower risk of sperm competition. Within satellites, larger males similarly obtain more mating opportunities and have larger testes. Their testes, however, are smaller proportionate to their body weight, even though all satellites experience the same risk of sperm competition. Within sneakers, testes weight increases proportionately with body weight. However, larger sneakers experience the same risk of sperm competition but obtain fewer mating opportunities. Thus, sneaker testes size does not seem to relate to either the number of mating opportunities or the risk of sperm competition.

Some of these apparent discrepancies in sperm investment patterns among individuals within a mating tactic may relate to the intensity of sperm competition. For example, large satellites may better mimic females and obtain superior

spawning positions, thereby giving their sperm a competitive advantage. Conversely, small sneakers may be more cryptic and obtain the superior spawning positions (Gross, 1980). These differences in mating position can lead to a "loaded raffle" mechanism that selects for reduced investment in sperm by larger satellites and smaller sneakers (Parker, 1990b). Differences in sperm characteristics, such as length or speed, that increase competitiveness (Balshine et al., 2001) can also lead to a loaded raffle. However, variation in sperm length has only been found in sneakers (Leach and Montgomerie, 2000). Thus, although differences in sperm competition intensity might contribute to the investment patterns within sneakers and satellites, it remains unclear why larger parentals invest proportionately more into sperm competition. No doubt further insight will come from closer examination of the gonad tissues, such as the histology and histochemistry (see Neat, 2001; Rasotto, 1995; Scaggiante et al., 1999).

Sperm competition risk does not appear to favor larger sperm in bluegill. It has been suggested that larger sperm may move faster and be more competitive (Balshine et al., 2001; LaMunyon and Ward, 1999; Stockley et al., 1997; for review, see Birkhead and Møller, 1998). These speculations, however, have largely been based on cross-species comparison. There is no evidence that these differences exist within species between alternative mating tactics (Gage et al., 1995, 1998; Schärer and Robertson, 1999). Given that cuckolders experience a greater risk of sperm competition than do parentals, cuckolder sperm might be expected to be longer if longer sperm are in fact more competitive. However, Leach and Montgomerie (2000) found no difference in sperm length between cuckolders and parentals. We found similar results based on a small sample (data not shown). Leach and Montgomerie did find that sperm length varied among individuals within a tactic, with smaller sneakers and possibly parentals in better condition having longer sperm. These differences do not relate to the risk of sperm competition because parentals in better condition obtain superior positions within mating colonies and thereby actually experience a lower risk of sperm competition, and all sneakers experience the same risk of sperm competition.

No clear model exists to explain variation in sperm longevity among individuals. It is generally thought that a trade-off between sperm number (or speed) and longevity will result in an inverse association between the risk or intensity of sperm competition and sperm longevity (Levitan, 2000; Parker, 1993; Petersen and Warner, 1998). However, longer-lived sperm have been found in the sneaker-type males that generally experience higher sperm competition risk in three-spined stickleback (de Fraipont et al., 1993), Atlantic salmon (Gage et al., 1995), and corkwing wrasse (Uglem et al., 2001). Conversely, we found that in bluegill, parental sperm live longer than do both sneaker and satellite sperm. Leach and Montgomerie (2000) also found that parental sperm live longer compared with that of sneakers, but their difference was not significant. Thus, longer-lived sperm does not appear to be a characteristic exclusive to either the sneakerlike or guardlike tactic.

Recent insights into the proximate mechanisms underlying sperm longevity might help to direct models of sperm longevity and competition. For example, seminal fluid can play an important role in sperm longevity. Ejaculates rich in mucins and other nutrients can prolong sperm longevity, even in aqueous environments common to fish (Marconato et al., 1996; Scaggiante et al., 1999). Thus, in bluegill, parentals may produce ejaculates rich in nutrients, thereby increasing sperm longevity. This could potentially allow parentals to decouple spawning with fertilization. For example, a parental occasionally breaks off spawning to chase a sneaker away from his nest. If the female releases eggs during this time, they could go unfertilized. However, sperm previously released by the parental in nutrient-rich seminal fluid could still be active in the nest with the potential to fertilize the eggs.

We found that cuckolders have greater ejaculate sperm density than do parentals. This is consistent with a previous study in bluegill (Leach and Montgomerie, 2000) and findings in bluehead wrasse (Schärer and Robertson, 1999), in which the cuckolder-like initial-phase males have greater ejaculate sperm density than did the parental-like terminal-phase males. The higher sperm density of cuckolders may be owing to the limitation in their body cavity for sperm storage (Gross, 1982) or selection for the ejaculation of sperm at a greater rate when under sperm competition (Schärer and Robertson, 1999). Within cuckolders, satellites have greater ejaculate sperm density than do sneakers, possibly because satellites require a tighter packaging of sperm to meet the demands of the threefold increase in mating opportunities that they obtain relative to sneakers. Because satellites fertilize fewer eggs than do sneakers when in competition with parentals (Fu et al., 2001), but may have superior mating position during oviposition, satellites likely release fewer sperm than do sneakers and therefore considerably less ejaculate per mating.

Based on the artificial competition experiments between cuckolder and parental ejaculates, we found that fertilization success is directly related to the relative number of sperm used. This is a common assumption used in raffle models of sperm competition (see Parker, 1990a,b, 1998). Interestingly, parental sperm appear better able at fertilizing eggs compared with cuckolder sperm, at least when equal volumes of milt are mixed and placed in direct contact with the eggs. It requires about 1.7 times more cuckolder sperm as parental sperm to obtain equal paternity. Given that cuckolders fertilize a greater proportion of eggs in nature when in direct competition with parentals (Fu et al., 2001), they must either obtain a better mating position during ejaculation or release considerably more sperm, as has been shown in other fishes (Alonzo and Warner, 2000; Shapiro et al., 1994). Within bluegill, mating positions during ejaculation and the volume of ejaculate released in nature remains to be explored.

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